Determining Stress Tolerance of *H. dujardini* Subjected to Extreme Conditions

Nicole Valentine, Luis Rivera, Kathleen Engelmann
UB Department of Biology
University of Bridgeport, Bridgeport, CT

Background

Tardigrades, commonly known as “water bears”, are small invertebrate animals. They range in Size from 0.1 to 1.5 mm In length. They are bilaterally symmetrical and segmented, having 4 pairs of legs, each with 4 to 8 claws at the ends. Tardigrades reproduce sexually, and asexually in a process known as parthenogenesis. Their diets consist of bacteria and plant and animal cell fluids. Typical environments in which they are found include, terrestrial (on moss and lichen), marine, and freshwater habitats.

Figure 1: Scanning electron micrograph of tardigrade

They are known for their capabilities to survive in a wide range of extreme conditions, including the vacuum of space, ionizing radiation, complete desiccation, and extreme temperatures. Tardigrades have been found to survive 30 years at −20°C, a few days at −200°C, a few minutes at −272°C, a few minutes at 151°C, and up to 87,022 psi. In addition, tardigrades are suspected to partake in horizontal gene transfer, and it is possible that up to one-sixth of their genome is made up of foreign DNA sequences.

Hypothesis

Based on tardigrade source, *H. dujardini* obtained from Carolina® lab**, i**t is hypothesized that they will have a low tolerance to radiation & extreme temperatures; and unlikely they will be capable of entering a “tun” state. In the wild, natural selection plays a role in selecting for individuals that are more resilient to changing conditions and therefore more reproductively successful. Such conditions may not be present in the lab.

Modes of Survival

Tardigrades can survive extreme conditions by entering a “tun” state. In this state, their body volume reduces up to 87% and metabolic activity reduces as low as .01%. Trehalose helps preserve cellular components. Tardigrades have been found to survive decades in a “tun”.

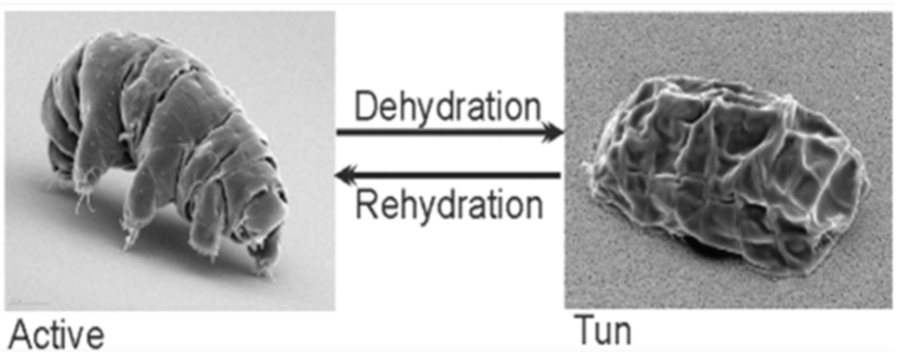

Figure 2: Scanning electron micrograph of active tardigrade versus tun state.

Methods

Step 1: **Normal Conditions (20°C)**	1. Place tardigrades in a concave slide with 1mL *Chlamydomonas reinhardi* at **20°C** in humidity chamber. 2. Supply 1mL algae culture 1x week. 3. Record the survival rate after 1hr treatment, then again after 1hr, 24 hours, and 48 hours.
Step 2: **UV Radiation**	1. Place tardigrades in a concave slide with 1mL *Chlamydomonas reinhardi* and irradiate with UV lamp. 2. Supply 1mL algae culture 1x week. 3. Record the survival rate after 1hr treatment, then again after 1hr, 24 hours, and 48 hours.
Step 3: **High Temperature (100°C)**	1. Place each in concave slide w/ 1mL *Chlamydomonas reinhardi* in oven at **100°C.** 2. Supply 1mL algae culture 1x week. 3. Record the survival rate after 1hr treatment, then again after 1hr, 24 hours, and 48 hours.
Step 4: **Low Temperature (-20°C)**	1. Place each in concave slide w/ 1mL *Chlamydomonas reinhardi* in freezer at **-20°C.** 2. Supply 1mL algae culture 1x week. 3. Record the survival rate after 1hr treatment, then again after 1hr, 24 hours, and 48 hours.
Step 5:	1. Determine overall survival rate for each group under each stressor.
Step 6:	1. Determine which group has the highest overall survival rate and under which conditions.

Table 1: Methods. *Chlamydomonas reinhardi* is the algae culture suppled for tardigrade’s food source.

Results

The low temperature group was the most successful with an overall % survival rate of 42%. The eggs of the control and the low temperature groups were able to survive treatment and their juveniles thrived. This can be seen in the data when the number of control individuals alive increases from 18 to 19 and 19 to 23 and when number of low temperature group alive increases from 3 to 5. All instances were in correlation with the number of eggs present decreasing.

The UV group was the only one that entered a tun state, as seen in Fig 5. After the 1 hour treatment, 22 of the 30 alive were in a tun. In addition, 1 hour after the treatment, 3 came out of the tun stage and were once again active.

The high temperature group were unable to survive, as seen in Fig 4. This is likely due to desiccation, rather than high temperature. A procedure was performed to test this hypothesis, and 0% of all groups survived desiccation.

Treatments	Control	UV Radiation	High Temp 100°C	Low Temp -20°C
Before Treatment	21	32	21	12
After 1 hr Treatment	18	30	0	3
1hr After Treatment	18	22	0	3
24 hrs After Treatment	19	17	0	5
48 hrs After Treatment	23	6	0	5
Overall % Survival Rate	23%	19%	0%	42%

Table 2: Results. Numbers represent number of individuals alive; active or tun state.

Figure 3: Control tardigrade before treatment (Active). *100x*

Figure 4: High temp. tardigrades after 1 hour treatment (Dead). *100x*

Figure 5: UV tardigrades after 1 hour treatment (Tun). *40x*

Conclusion

Although the specimens tested were lab cultured rather than gathered from the wild, they retained their remarkable ability to survive in extreme conditions.

All of the groups of tardigrades tested were much more successful than hypothesized, with the exception of the individuals that underwent high temperature treatment. The tardigrades obtained from Carolina® lab were capable of withstanding extreme conditions of low temperature and UV radiation; and, subsequently, able to reproduce and thrive thereafter. In addition, individuals that were exposed to UV radiation were unexpectedly capable of forming a tun.

To test whether the lab purchased specimens are as resilient as wildly cultivated ones, further experimentation could be done to compare their tolerance to extreme conditions. Although this was attempted, it was not successful due to inability to find wild specimens.

References

"Protozoa and Invertebrate Manual." *Protozoa and Invertebrate Manual Carolina.com*. N.p., n.d. Web. 10 Dec. 2016.

"Tardigrades Care Sheet." *Tardigrades Care Sheet Carolina.com*. N.p., n.d. Web. 10 Dec. 2016.